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(54) Title: METHOD OF DIAGNOSING NEURODEGENERATIVE DISEASE

(57) Abstract: The present invention provides methods for diagnosing neurodegenerative disease such as Alzheimer's disease, monitoring the progression and prognosis of the disease and/or monitoring the therapeutic efficacy of any intervention or treatment of the disease comprising measuring the level of α7 nicotinic acetylcholine receptor protein.

METHOD OF DIAGNOSING NEURODEGENERATIVE DISEASE

Background of the Invention

Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) afflict humanity with great suffering and financial loss. AD is characterized by neurofibrillary tangles, neuritic plaques, and neuronal cell death. AD appears as either the familial, early onset or late-onset forms, with the latter being more prevalent. AD is the major cause of age-related dementia and cognitive impairment (Wisniewski, T.; Ghiso, J.; Frangione, B. *Neurobiol. of Disease* 1997, *4*, 313-328). Currently the only effective diagnostic method consists of cognitive function of the patient. There is a need to develop sensitive, biochemical methods to determine the prognosis, progression, and monitor therapeutic efficacy of subjects likely to develop or suffering from Alzheimer's disease.

The neurotoxic β-amyloid peptide$_{1-42}$ [$A\beta_{1-42}$] is abundantly present in the amyloid plaques of Alzheimer's disease (AD) brains and also modulates cholinergic functions which are critical in memory and cognitive neurophysiology (Auld, D.S., Kar, S., Quirion, R. *Trends Neurosci* 1998; 21: 43-49). $A\beta_{1-42}$ interacts selectively and with high affinity to the neuronal pentameric cation channel α7 nicotinic acetylcholine receptor (Wang, H.-Y. et al. *J. Biol. Chem.* 2000, in press). This peptide-receptor interaction is likely neurotoxic due to the observation that stimulation of the alpha-7 subtype of the nicotinic acetylcholine receptors (nAChRs) can protect neurons against Aβ cytotoxicity (Kihara, T. et al. *Ann. Neurol.* 1997, *42*, 159). Also, compounds that activate nAChRs, especially of the alpha-7 subtype, have been found to have *in vivo* activity in models of cognition enhancement (US Patent No. 5,741,802, issued April 21, 1998). The α7 nicotinic acetylcholine receptor is involved in calcium homeostasis and modulation of

acetylcholine release in the synapses, all of which are critical events leading to memory formation. Beta-amyloid peptide increases cytosolic-free Ca^{2+} in AD lymphoblasts (Ibarreta et al., *Alzheimer Dis Assoc Disord* 1997 Dec; 11(4):220-7), and elevates mitogen-induced Ca^{2+} responses in freshly prepared human lymphocytes (Eckert et al., *Life Sci* 1994;55(25-26):2019-29). Amyloid precursor protein (APP) can be induced on the cell surface of human lymphocytes upon stimulation (Bullido et al., *Biochim Biophys Acta* 1996 Aug 21;1313(1):54-62) and increased APP-770 isoform occurs in lymphocytes from AD patients (Ebstein et al., *Brain Res Mol Brain Res* 1996 Jan;35(1-2):260-8). Lymphoblastoid cells from patients with early-onset and late-onset familial AD show increased expression of APP mRNA and protein (Matsumoto et al., *Eur J Biochem* 1993 Oct 1;217(1):21-7). The interaction of $A\beta_{1-42}$ and the α7 nicotinic acetylcholine receptor may initiate a series of pathophysiological events observed in AD leading to neurotoxicity and contribute to cognitive dysfunction, a hallmark of ADInvestigators have measured α7 nicotinic acetylcholine receptor mRNA levels in normal and AD subjects' brains in order to determine changes in regional distribution or changes in the total amount of mRNA levels between the subject populations. α7 nicotinic acetylcholine receptor mRNA was equally distributed in all areas of the brain except the hippocampus, where AD subjects exhibited higher mRNA levels than the control population. Similarly, lymphocytes from AD patients exhibit an increased mRNA level for α 7 nicotinic acetylcholine receptor (Hellstrom-Lindahl et al., *Brain Res Mol Brain Res* 1999 Mar 20;66(1-2):94-103). In a second report, nicotinic acetylcholine receptor binding in brains of AD and control subjects showed no significant difference (Lang, W. and Henke, H. *Brain Res* 1983; 267: 271-280). The present invention provides the unexpected observation that α7 nicotinic acetylcholine receptor protein levels are decreased in neuronal tissue, thus forming the basis of a biochemical assay to diagnose Alzheimer's disease.

Summary of the invention

The present invention provides methods for diagnosing Alzheimer's disease, monitoring the progression and prognosis of Alzheimer's disease and/or monitoring the therapeutic efficacy of any intervention or treatment of Alzheimer's disease comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein;

(b) contacting the test sample with a compound capable of specific interaction with the α7 nicotinic acetylcholine receptor protein; and

(c) measuring the binding of the compound to α7 nicotinic acetylcholine receptor protein as an indication of α7 nicotinic acetylcholine receptor protein in the test sample.

Brief description of the drawings

Figure 1. α7 nicotinic acetylcholine receptor protein levels in Alzheimer's disease (AD) brains are reduced. Equal amounts of hippocampal proteins from AD or control subjects were subjected to electrophoresis and Western blot analysis using anti-α7 nicotinic acetylcholine receptor antibodies. The intensity of the bands was measured by densitometry. Representative data from three AD [A1-3] and control [C1-3] are shown. Molecular weight markers in kiloDalton are shown.

Detailed description of the invention

The present invention also provides tools useful for diagnosing Alzheimer's disease. Among other tissues, Alzheimer's disease (AD) exhibits neuropathological abnormalities in the olfactory system

located in the nasal cavity. These include the presence of dystrophic neurites that exhibit immunoreactivity for tau, neurofilaments, apolipoprotein E and other proteins, abnormal tau protein, increase in superoxide dismutase, and ß amyloid deposition in the primary sensory (olfactory receptor) cells and nerve fibres of the nasal mucosa tissue (Arnold et al., Ann N Y Acad Sci 1998 Nov 30;855:762-75; Hock et al., *Eur Neurol* 1998 Jul;40(1):31-6; Johnson et al., *Neurobiol Aging* 1994 Nov-Dec;15(6):675-80; Kulkarni-Narla et al., *Exp Neurol* 1996 Aug;140(2):115-25; Lee et al., *Exp Neurol* 1993 May;121(1):93-105; Tabaton et al., Neurology 1991 Mar;41(3):391-4; Talamo et al., Ann N Y Acad Sci 1991;640:1-7; Yamagishi et al., *Ann Otol Rhinol Laryngol* 1998 May;107(5 Pt 1):421-6; Yamagishi et al., *Nippon Jibiinkoka Gakkai Kaiho* 1994 Jan;97(1):51-60). These observations recapitulate the neuropathological profile and neurodegenerative abnormalities (e.g., cytoskeletal changes, protein immunoreactivity and ß amyloid deposition) observed in central nervous system neurons from AD patients. Routine access to these sensory neurons and fibers can be done with nasal biopsy in AD patients (e.g., Feron et al., *Arch Otolaryngol Head Neck Surg* 1998 Aug;124(8):861-6).

Olfactory neuroblasts (olfactory neurons obtained by biopsy and placed in primary cell culture) from AD patients produce carboxyl terminal amyloid precursor protein (APP) fragments that contain ß amyloid (Aß)(Crino et al., *Ann Otol Rhinol Laryngol* 1995 Aug;104(8):655-61). Crino et al. showed labeling of Aß in the basal third of the olfactory neuroepithelium and in axons projecting through the lamina propria of AD patients. Thioflavin-S staining that detects amyloid deposition was also observed in the basal third of the olfactory neuroepithelium from AD patients. α7 nicotinic acetylcholine receptors are present in olfactory neurons probably including olfactory receptor cells in the nasal cavity (Alkondon et al., *Neurosci Lett* 1994 Aug 1;176(2):152-6; Alkondon et al., *Eur J Neurosci* 1997 Dec;9(12):2734-42; Bouvet et al., *Neurosci Res* 1988

Feb;5(3):214-23; Edwards et al., *Experientia* 1987 Aug 15;43(8):868-73; Edwards et al., *Experientia* 1988 Mar 15;44(3):208-11; Seguela et al., *J Neurosci* 1993 Feb;13(2):596-604).

Lymphocytes (including B cells, T cells) and neutrophils, also likely express α7 nicotinic acetylcholine receptor protein, though evidence of such expression is indirect via the observation of Beta-amyloid peptide functional activity in AD patients (Ibarreta et al, Ekert et al) or by measurement of mRNA (Hellstom-Lindahl et al). The inventors contemplate that numerous clinically available tissues express α7 nicotinic acetylcholine receptor, which can be easily determined by a method comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell;

(b) contacting the test sample with a compound capable of specific interaction with the α7 nicotinic acetylcholine receptor; and

(c) measuring binding of the compound to the test sample.

The inventors further contemplate that the α7 nicotinic acetylcholine receptor protein may be increased in some tissues or cell types, while decreased in others. Further, the inventors contemplate that α7 nicotinic acetylcholine receptor protein may be sequestered within the cell, and that intracellular α7 nicotinic acetylcholine receptor protein may be increased in AD subjects. As previously noted, the quantity of mRNA is apparently distinct from the protein levels in any particular cell type.

The analysis of the changes in the level of α7 nicotinic acetylcholine receptor protein in lymphocytes and olfactory neuroepithelial neurons/neuronal processes or olfactory neuroblasts obtained from AD patients can be used as an AD diagnostic marker, useful for AD prognosis, progression, and therapeutic efficacy of any intervention or treatment targeting AD. The present invention provides the unexpected observation that the α7 nicotinic

acetylcholine receptor protein is down-regulated in hippocampal tissue of AD subjects, rather than unregulated, as has been observed in the mRNA levels for the α7 nicotinic acetylcholine receptor gene.

The present invention provides methods for diagnosing Alzheimer's disease, monitoring the prognosis and progression of Alzheimer's disease and monitoring the therapeutic efficacy of any intervention or treatment of Alzheimer's disease comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein;

(b) contacting the test sample with a compound capable of specific interaction with the α7 nicotinic acetylcholine receptor protein; and

(c) measuring the binding of the compound to α7 nicotinic acetylcholine receptor protein as an indication of α7 nicotinic acetylcholine receptor protein in the test sample.

A "sample" as used herein, refers to any substance that may contain α7 nicotinic acetylcholine receptor protein. A sample can be biological fluid, such as whole blood or whole blood components including red blood cells, white blood cells, platelets, serum and plasma, ascites, urine, cerebrospinal fluid, and other constituents of the body that may contain the cell or fragment containing α7 nicotinic acetylcholine receptor protein. Further, a sample may be a component in a larger composition, for example in a tissue section of a biopsy, where the cells of interest may belong to one or more cellular subtypes amongst a field of different cell types.

The term "cell" refers to at least one cell, but includes a plurality of cells, or fractions of cells appropriate for the sensitivity of the detection method. Cells suitable for the present invention may be present as isolated, purified cell populations or as a fraction of an organized tissue biopsy. Fractions of cells,

including the axon or dendrites of a neuron are also suitable for use in the present invention, and may be isolated, for example in a tissue section of a biopsy. Preferred cells suitable for use in the present invention are selected from the group consisting of circulating lymphocytes, olfactory neuroepithelial neuronal cell bodies or their neuronal processes, or hippocampal cells.

The phrase "compound capable of specific interaction with the α7 nicotinic acetylcholine receptor" refers to, for example, synthetic or natural amino acid polypeptides, proteins, small synthetic organic molecules, or deoxy- or ribo- nucleic acid sequences that bind to the α7 nicotinic acetylcholine receptor with about 20-fold or greater affinity compared to other proteins. For example, but not by way of limitation, $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{1-43}$, peptides, purified from natural sources or created synthetically using peptide synthetic methods, polyclonal or monoclonal antibodies raised against the α7 nicotinic acetylcholine receptor, or a peptide fragment thereof, or small organic molecules that block $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{1-43}$ peptide interaction with the receptor are suitable for use in the present invention. Preferred compounds of the present invention include $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{1-43}$ peptides, or antibodies that bind to the a7 nicotinic acetylcholine receptor. Compounds useful in the present invention may be labeled compounds, with means of direct detection, or may be detected by an indirect means, for example by a second labeled compound.

The phrase "labeled compound" refers to moieties capable of measurement comprising radioactive atoms, enzymes, fluorescent molecules, or alternative tags, for example biotin. Particular radioisotopes useful as a label in the present invention are ^{3}H, ^{125}I, ^{131}I, ^{35}S, ^{32}P, or ^{33}P and others that can be applied in brain imaging analyses. Particular examples of enzymes suitable for use in the present invention are horseradish peroxidase, alkaline phosphatase, or luciferase. A preferred example of a detectable label is an enzyme that cleaves a substrate to yield a chromogenic

or luminescent product. Particular examples of fluorescent molecules are fluorescein (FITC), rhodamine, or Alexa™ dyes (Molecular Probes). Direct measurement is conducted by observing the presence of the radioactive atom or flourogenic molecule, or by observation of enzymatic activity of a colorimetric or luminescent substrate. Indirect measurement is conducted by adding an additional compound including a label to the test sample so that it can interact with the compound bound to the test sample. A well-known example is when the labeled compound comprises biotin, and a second compound comprises avidin or streptavidin and a detectable label. A second well-known example is when a first antibody is used to bind to the α7 nicotinic acetylcholine receptor and is detected with a second anti-antibody comprising a detectable label. In this case the first antibody comprises a label in that there are specific regions capable of detection within the structure of the first antibody.

The level of α7 nicotinic acetylcholine receptor protein can be assessed by measuring its direct binding to the labeled compound. Alternatively the level of α7 nicotinic acetylcholine receptor protein can be assessed by measuring the binding of labeled α7 nicotinic acetylcholine receptor protein or an analogue which competes for binding with α7 nicotinic acetylcholine receptor protein to an unlabeled compound that interacts with α7 nicotinic acetylcholine receptor protein. These and other methods for immunoaffinity or ligand affinity measurements are well known to those skilled in the art.

The method of the present invention can be further defined by adding a step (d) of comparing changes in the level of α7 acetylcholine protein in the test sample with an established normal level determined by measuring the α7 nicotinic acetylcholine receptor protein in an normal samples. "Normal sample" as used herein refers to a sample from a subject who demonstrates no detectable neurodegenerative disease, for

example, Alzheimer's disease, by known cognitive diagnostic methods, and may include preserved tissue sections from tissue archives. The measurement means of step (c) suitable for the method of the present invention comprises measuring changes in the quantity of intracellular protein, or cell surface protein. Immunoaffinity or ligand affinity measurement quantitates levels of protein in or on the surface of host cells. Unlabelled α7 nicotinic acetylcholine receptor protein is detected by Western blotting, cell surface detection by fluorescent cell sorting, cell image analysis, and immunoassay employing compounds specific for α7 nicotinic acetylcholine receptor protein. Preferred detection means for cell surface protein include flow cytometry or statistical cell imaging. In both techniques the protein of interest is localized at the cell surface, labeled with a specific fluorescent probe, and detected via the degree of cellular fluorescence. In flow cytometry, the cells are analyzed in a solution, whereas in cellular imaging techniques, a field of cells is compared for relative fluorescence.

The methods of the present invention may be used to measure intracellular α7 nicotinic acetylcholine receptor protein contained within the entoplasmic reticulum, Golgi complex, or vesicles. This method provides that the test sample be pretreated to perforate the cell membranes or to solubilize the cell, for example with a detergent containing solution, both techniques are well known in the art. When the cell membranes are perforated, the compound enters the cell and specifically interacts with the α7 nicotinic acetylcholine receptor in the compartments previously described. Cell extracts provide soluble α7 nicotinic acetylcholine receptor protein that may be measured by the methods described herein, preferably by ELISA utilizing a chemiluminescent detection system. The method to detect intracellular α7 nicotinic acetylcholine receptor protein comprises the steps:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein in healthy individuals;

(b) contacting the test sample with a membrane perforating agent;

(c) contacting the test sample with a labeled compound capable of specific interaction with the α7 nicotinic acetylcholine receptor; and

(d) measuring the binding of the compound to α7 nicotinic acetylcholine receptor protein as an indication of α7 nicotinic acetylcholine receptor protein in the test sample.

Membrane perforating agents are well known in the art, and are used, for instance to perforate lymphocytic cells to analyze intracellular components using flow cytometry. These agents are commercially available, for example, by Ortho Clinical Diagnostics under the name PERMEAFIX™.

A particular assay of the present invention comprises binding protein assay methods using a compound capable of specific interaction with α7 nicotinic acetylcholine receptor protein to detect changes in the level of α7 nicotinic acetylcholine receptor protein. Preferably the compound is an antibody to α7 nicotinic acetylcholine receptor protein. The preferred method to detect α7 nicotinic acetylcholine receptor protein comprises the steps:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein in healthy individuals;

(b) contacting the test sample with a labeled antibody capable of specific interaction with the α7 nicotinic acetylcholine receptor, wherein the antibody becomes a bound antibody or an unbound antibody;

(c) removing the unbound antibody from the test sample; and

(d) measuring the quantity of bound or unbound antibody to indicate changes in the level of α7 nicotinic acetylcholine receptor protein in the test sample.

Particular changes in the level of the α7 nicotinic acetylcholine receptor protein are either to increase or to decrease in the test sample. The preferred change in the level of the α7 nicotinic acetylcholine receptor protein in lymphocytes, olfactory tissue, and hippocampal tissue is to decrease as an indication of development or worsening of AD in the subject.

The following examples illustrate the present invention without, however, limiting the same thereto.

EXAMPLE 1

Western blot analysis of α7 nicotinic acetylcholine receptor

We analyzed 12 sporadic AD brains (mean age = 73.9 ± 1.5, mean postmortem time interval = 11.7 ± 1.4 hr) from subjects that were clinically diagnosed according to the criteria defined by The National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of AD (The National Institute on Aging and Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease. *Neurobiol. Aging* 1997; **18(4 Suppl.)**: S1-2). These tissues were further characterized by immunohistochemistry to verify the amyloid plaques and neurofibrillary tangles pathology. We compared the protein levels of the α7 nicotinic acetylcholine receptor in these AD brains to those of the control non-demented subjects (n = 12, mean age = 71.6 ± 1.4, mean postmortem time interval = 10 ± 2.0 hr). Equal amounts of hippocampal total protein

were subjected to electrophoresis, Western blotting and analysed using anti-α7 nicotinic acetylcholine receptor antibodies (RBI, Fig. 1). A horseradish peroxidase conjugated secondary antibody was employed in the chemiluminscence detection protocol (Supersignal, Pierce & Warriner). Figure 1 shows that the amount of α7 nicotinic acetylcholine receptor protein was reduced. The extent of the reduction was measured by densitometry and indicated that the α7 acetylcholine receptor was reduced by 57±5% compared to controls (Table 1, $p < 0.04$). A reduction was observed in all twelve AD brain samples tested.

Table 1

Postmortem brain

	Diagnosis	Age	Gender	Post Mortem interval (hr)
A1	AD	78	Female	9.4
A2	AD	72	female	17.7
A3	AD	73	female	14.1
A4	AD	76	female	15.5
A5	AD	73	female	3
A6	AD	77	female	15.3
A7	AD	78	male	6.7
A8	AD	75	female	17.5
A9	AD	77	male	6.6
A10	AD	79	male	14.9
A11	AD	61	male	7.3
A12	AD	68	male	12.6
C1	Control	70	female	18.4
C2	Control	69	male	9.8
C3	Control	70	male	14.9
C4	Control	70	male	14.3
C5	Control	76	male	5.4
C6	Control	81	male	24
C7	Control	68	male	4.1
C8	Control	77	male	2.3
C9	Control	68	male	12
C10	Control	70	male	6.3
C11	Control	64	female	5.4
C12	Control	75	female	3.5

Postmortem brains were obtained from Harvard Brain Tissue Resource Center at McLean Hospital (Belmont, MA) and Analytical Biological Services, Inc.(Wilmington, DE). The mean ages of the subjects for AD and controls were 73.9 ± 1.5 and 71.6 ± 1.4, respectively. The postmortem interval for AD and control subjects were 11.7 ± 1.4 hr and 10.0 ± 2.0 hr, respectively.

WHAT IS CLAIMED IS:

1. A method of diagnosis, determining prognosis, progression, or monitoring therapeutic efficacy of any intervention or treatment of neurodegenerative disease comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein;

(b) contacting the test sample with a compound capable of specific interaction with the α7 nicotinic acetylcholine receptor protein; and

(c) measuring the binding of the compound to α7 nicotinic acetylcholine receptor protein as an indication of α7 nicotinic acetylcholine receptor protein in the test sample.

2. The method of claim 1 wherein the neurodegenerative disease is Alzheimer's disease.

3. The method of claim 1 wherein the compound is a labeled compound.

4. The method of claim 1, 2 or 3 further comprising adding a step (d) of comparing changes in the level of α7 nicotinic acetylcholine receptor protein in the test sample with a normal sample from a subject known to lack neurodegenerative disease.

5. The method of claim 1, 2 or 3 wherein the test sample comprises a cell selected from the group consisting of circulating lymphocytes, olfactory neuroepithelial neuronal cell bodies or their neuronal processes, and hippocampal cells.

6. The method of claim 1 or 2 wherein the compound is selected from the group consisting of $A\beta_{1-40}$ peptide, $A\beta_{1-42}$ peptide, $A\beta_{1-43}$ peptide, a monoclonal antibody, and a polyclonal antibody.

7. The method of claim 1, 2, or 3 wherein the change is a decrease in the level of the α7 nicotinic acetylcholine receptor protein.

8. A method of diagnosis, determining prognosis, progression, or monitoring the therapeutic efficacy of any intervention or treatment of Alzheimer's disease comprising:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell selected from the group consisting of circulating lymphocytes, olfactory neuroepithelial neuronal cell bodies or their neuronal processes, or hippocampal cells, said cell expressing α7 nicotinic acetylcholine receptor protein in healthy individuals;

(b) contacting the test sample with an antibody capable of specific interaction with the α7 nicotinic acetylcholine receptor protein; and

(c) measuring the binding of the antibody to the α7 nicotinic acetylcholine receptor protein as an indication of decreases in the level of α7 nicotinic acetylcholine receptor protein in the test sample.

9. The method of claim 8 further comprises adding a step (d) of comparing changes in the level of α7 acetylcholine receptor protein in the test sample with a normal sample, from a subject known to lack neurodegenerative and neurological diseases.

10. A method to detect intracellular α7 nicotinic acetylcholine receptor protein in an Alzheimer's Disease subject which comprises the steps:

(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein in healthy individuals;

(b) contacting the test sample with a membrane perforating agent;

(c) contacting the test sample with a labeled compound capable of specific interaction with the α7 nicotinic acetylcholine receptor; and

(d) measuring the binding of the compound to α7 nicotinic acetylcholine receptor protein as an indication of α7 nicotinic acetylcholine receptor protein in the test sample.

11. An immunoassay method to detect α7 nicotinic acetylcholine receptor protein in an Alzheimer's Disease subject which comprises the steps:

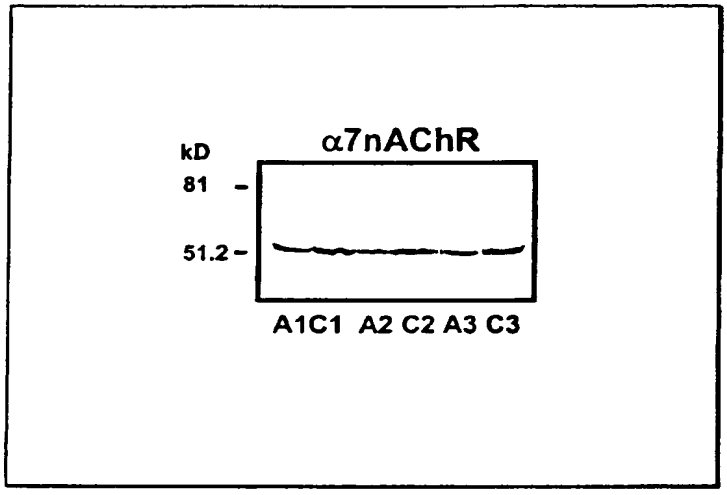
(a) obtaining a test sample from a subject wherein the test sample comprises a cell, said cell expressing α7 nicotinic acetylcholine receptor protein in healthy individuals;

(b) contacting the test sample with a labeled antibody capable of specific interaction with the α7 nicotinic acetylcholine receptor, wherein the antibody becomes a bound antibody or an unbound antibody;

(c) removing the unbound antibody from the test sample; and

(d) measuring the quantity of bound or unbound antibody to indicate changes in the level of α7 nicotinic acetylcholine receptor protein in the test sample.

FIGURE 1



INTERNATIONAL SEARCH REPORT

Interna al application No.
PCT/US00/31467

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 2/00, 14/00, 16/00, 17/00; C12Q 1/00; G01N 33/53, 33/566, 33/567
US CL : 435/4, 7.1, 7.2; 436/501, 503; 530/300, 350, 387.1, 388.1, 388.22, 389.1, 391.1, 391.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/4, 7.1, 7.2, 436/501,503; 530/300, 350, 387.1, 388.1, 388.22, 391.1, 391.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, DIALOG (file biosci), MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WEVERS ET AL. Expression of nicotinic acetylcholine receptor subunits in the cerebral cortex in Alzheimer's disese: histotopographical correlation with amyloid plaques and hyperphosphorylated-tau protein. Eur. J. Neurosci., 1999, Vol. 11, No. 7, pages 2551-2565, especially pages 2555-2564.	1-11
X	ARAUJO ET AL. Differential Alteration of Various Cholinergic Markers in Cortical and Subcortical Regions of Human Brain in Alzheimer's Disease. J. Neurochem., 1988, Vol. 50, pages 1914-1923, especially pages 1915-1917 and 1919-1921.	1-5, 7, 9-10
X	SCHRODER ET AL. Nicotinic Cholinoceptive Neurons of the Frontal Cortex are Reduced in Alzheimer's Disease. Neurobiology. Aging, 1991, Vol. 12, pages 259-262, see entire document.	1-11
A	LEVIN, E.D. Nicotinic systems and cognitive function. Psychopharmacology., 1992, Vol. 108, pages 417-431, especially pages 424-425.	1-11
A	BREESE ET AL. Comparison of the Regional Expression of Nicotinic Acetylcholine Receptor alpha 7 mRNA and [I]-alpha-Bungarotoxin Binding in Human Postmortem Brain. J. Comp. Neurol., 1997, Vol. 387, pages 385-398, see entire document.	1-11

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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02 February 2001 (02.02.2001)	18 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Bridget E. Bunner Telephone No. (703) 308-0196

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Internal application No.
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C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COURT ET AL. Nicotinic and muscarinic cholinergic receptor binding in the human hippocamal formation during development and aging. Dev. Brain Res., 1997, Vol. 101, pages 93-105, especially pages 93-99 and 101-102.	1-11

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